

University of Groningen

Toxoplasmic encephalitis relapse rates with pyrimethamine-based therapy

Connolly, Mark P.; Goodwin, Elizabeth; Schey, Carina; Zummo, Jacqueline

Published in:
Pathogens and Global Health

DOI:
[10.1080/20477724.2016.1273597](https://doi.org/10.1080/20477724.2016.1273597)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Connolly, M. P., Goodwin, E., Schey, C., & Zummo, J. (2017). Toxoplasmic encephalitis relapse rates with pyrimethamine-based therapy: systematic review and meta-analysis. *Pathogens and Global Health*, 111(1), 31-44. <https://doi.org/10.1080/20477724.2016.1273597>

Copyright

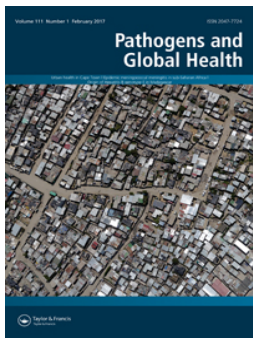
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.



Toxoplasmic encephalitis relapse rates with pyrimethamine-based therapy: systematic review and meta-analysis

Mark P. Connolly, Elizabeth Goodwin, Carina Schey & Jacqueline Zummo

To cite this article: Mark P. Connolly, Elizabeth Goodwin, Carina Schey & Jacqueline Zummo (2017) Toxoplasmic encephalitis relapse rates with pyrimethamine-based therapy: systematic review and meta-analysis, Pathogens and Global Health, 111:1, 31-44, DOI: 10.1080/20477724.2016.1273597

To link to this article: <https://doi.org/10.1080/20477724.2016.1273597>



© 2017 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group



[View supplementary material](#)



Accepted author version posted online: 05 Jan 2017.
Published online: 16 Jan 2017.



[Submit your article to this journal](#)



Article views: 1376



[View related articles](#)



[View Crossmark data](#)



Citing articles: 14 [View citing articles](#)



Toxoplasmic encephalitis relapse rates with pyrimethamine-based therapy: systematic review and meta-analysis

Mark P. Connolly^{a,b}, Elizabeth Goodwin^c, Carina Schey^{a,b} and Jacqueline Zummo^c

^aUnit of PharmacoEpidemiology & PharmacoEconomics, Department of Pharmacy, University of Groningen, Groningen, The Netherlands;

^bHealth Economics, Global Market Access Solutions LLC, Mooresville, NC, USA; ^cMedical Affairs, Turing Pharmaceuticals, New York, USA

ABSTRACT

Toxoplasmic encephalitis (TE) is caused by *Toxoplasma gondii* infection and can be a life-threatening disease in immunocompromised patients. This study evaluated the rate of relapse associated with pyrimethamine-based maintenance therapy (i.e. secondary prophylaxis) in patients with human immunodeficiency virus (HIV) or AIDs treated prior to and after the common use (i.e. 1996) of highly active antiretroviral therapy (HAART) (pre-HAART and post-HAART, respectively). PubMed, Google Scholar, and Cochrane databases were searched to 6 June 2016 using search terms: pyrimethamine, Daraprim, Fansidar, Metakelfin, Fansimef, 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine, encephalitis, cerebral, toxoplasmosis, toxoplasmic, and *gondii*. Single-arm cohort, retrospective, and randomized studies were included. Twenty-six studies with 1,596 patients were included in the analysis; twenty pre-HAART ($n = 1,228$) studies and six post-HAART ($n = 368$) were performed. Pooled proportions test for pyrimethamine-based therapy from pre-HAART studies indicated a relapse rate of 19.2% and 18.9% from the fixed-effects and random-effects models, respectively. The relapse rate in the post-HAART studies was 11.1% (fixed and random effects). Continuous therapy was suggestive of lower incidence of relapse compared with intermittent therapy in the pre-HAART era (range, 18.7 to 17.3% vs. 20.9 to 25.6%, respectively). These findings indicate that the likelihood of relapse associated with pyrimethamine-based therapy in patients with HIV and TE decreased after the introduction of HAART to approximately 11%. The findings have important implications as relapse may affect a patient's disease severity and prognosis, increase utilization of health care resources, and result in additional health care expenditure.

KEYWORDS

Toxoplasmosis; Encephalitis; Toxoplasma; Pyrimethamine; Human immunodeficiency virus; Meta-analysis; Relapse; Proportions

Introduction

Toxoplasmic encephalitis (TE) results from infection by *Toxoplasma gondii* (*T. gondii*), a ubiquitous obligate intracellular parasite with a worldwide prevalence that infects humans and other warm-blooded animals [1]. Approximately, one third of the population worldwide is chronically infected with *T. gondii* [2], *T. gondii* seroprevalence varies world wide. In the United States, it is estimated that 22.5% of the population 12 years and older are infected with the parasite [3]. It is estimated that 6.7% of people in Korea, 12.3% in China, 23.9% in Nigeria, 46% in Tanzania, and 47% in the rural areas of France are seropositive for *T. gondii* [4–8]. In Brazil, up to 50% of elementary school children and 50 to 80% of women of child-bearing age have antibodies to *T. gondii* [9]. *T. gondii* infection has two phases: active (acute) which is characterized by severe symptoms; and latent (dormant) which is typified by life-long persistence of cysts in tissues [10]. TE typically results from reactivation of the dormant organism [11].

TE is one of the most common opportunistic infections of the central nervous system (CNS) in patients with acquired immune deficiency syndrome (AIDS) worldwide, and in the United States between 10 and 40% of HIV-infected individuals have antibodies against *T. gondii* [12–14]. TE is a life-threatening disease, especially for immunocompromised patients, such as those with AIDS [13,15]. Encephalitis causes significant morbidity and mortality in patients with HIV, and toxoplasmosis was found to be the most common specified encephalitis-associated hospitalizations in patients with HIV [16].

The National Institutes of Health (NIH) Guidelines recommend the use of pyrimethamine, sulfadiazine, and leucovorin for the initial treatment of TE in HIV-infected patients [17]. Pyrimethamine and sulfadiazine are thought to act synergistically to inhibit *T. gondii* proliferation and survival by blocking the folate metabolic pathway and consequently DNA synthesis [11]. Leucovorin is added to reduce potential adverse reactions associated with folic acid metabolism [11]. In case of allergy to sulfa

CONTACT Mark P. Connolly m.connolly@rug.nl

Supplemental data for this article can be accessed [here](#).

© 2017 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

drugs, the recommended alternative treatment is a combination of pyrimethamine, clindamycin, and leucovorin [17]. Sulfa drug allergies are observed in about 5% of the population and up to 30% of HIV-infected patients [18].

The incidence of TE in patients infected with HIV is closely related to the progression of immune deficiency and a reduction in CD4 T lymphocyte cell counts. TE is rare in patients with CD4 cell counts >200 cells/ μ L, while the risk of TE is greatest in patients with CD4 counts <50 cells/ μ L [19]. It is recommended by the NIH/AIDS Guidelines to continue chronic maintenance treatment in patients who have responded to initial TE therapy until the person remains asymptomatic and their CD4 counts are >200 cells/ μ L after highly active anti-retroviral therapy (HAART) for six months [17].

Despite treatment efforts, a percentage of patients receiving maintenance therapy for TE will experience a relapse [13]. Relapse of TE can have significant implications for patients who often develop new lesions in areas of the brain previously free of infection [20], and for providers and the health care system as a relapse can result in rehospitalization and/or change in therapy.

Previous meta-analyses have assessed the efficacy and safety of pyrimethamine for acute TE [21–23]; however, the incidence of relapse during maintenance therapy following resolution of acute TE has not been assessed [21–24]. Therefore, to gain greater insight into the risk of TE relapse, we performed a systematic review and meta-analysis to evaluate the incidence of relapse associated with pyrimethamine-based maintenance therapy (i.e. secondary prophylaxis) in persons with HIV/AIDS. We also investigated whether the likelihood of relapse changed following the introduction of HAART therapy in the mid-1990s. This was of interest because HAART has greatly reduced AIDS-related morbidity and mortality and decreased the incidence of opportunistic infections in HIV-infected patients [25]. The impact of HAART on the incidence of relapse of TE in patients with HIV is not clear. In order to incorporate the broadest scope of available evidence, the analysis protocol allowed for inclusion of studies of varying design, such as single-arm observational cohort, randomized, and retrospective studies.

Methods

Search strategy

The study was performed in accordance with PRISMA [26], and the protocol can be found in Supplemental Material. PubMed, Google Scholar and Cochrane databases were searched up to 6 June 2016 using the following search terms: pyrimethamine, Daraprim, Fansidar, Metakelfin, Fansimef, 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine, encephalitis, cerebral, toxoplasmosis, toxoplasmic, *gondii*. Eligible studies included randomized controlled studies, observational prospective studies, and

retrospective longitudinal studies. Studies had to report relapse of TE during maintenance therapy in patients with AIDS or HIV. All studies had to be published in peer-reviewed journals in English, Spanish, Portuguese, or French. Studies that only provided relapse rates during acute treatment were rejected. Review articles, letters, comments, editorials, case reports, proceedings, personal communications, and preclinical studies were also excluded. Study eligibility for inclusion was determined by two independent reviewers, and in cases of uncertainty, a third reviewer was consulted.

Data extraction

Double data extraction was performed on all included studies to ensure data consistency. Discrepancies in extracted data between the two independent reviewers were resolved by a third reviewer. Non-English studies were reviewed by native speakers, and validated by a second reviewer for extracted data using Google Translator. The following data were extracted from the studies when reported: first author, publication year, study design, acute and maintenance treatment regimens, number of patients, incidence of relapse, and duration of follow-up. For studies with insufficient reported data, we attempted to contact authors to obtain complete data. Studies were classified as being pre-HAART or post-HAART based on a cut-off date of 1996 for the conduct of the study (not publication date). The year 1996 is when the use of HAART therapy became widely available and dramatically decreased mortality of patients with AIDS (from 29.4/100 person years in 1995 to 8.8 per 100 person-years in the second quarter of 1997) [25]. HAART status was verified by patient baseline characteristics and current treatments reported, although not all patients treated in the HAART period were receiving antiretroviral therapy. The abstracts were screened and data were extracted by the authors MPC and EG.

Quality assessment

The quality of the included studies was evaluated using Study Quality Assessment Tools of the National Heart, Lung, and Blood Institute of the NIH for quality assessment of observational cohort, cross-sectional, before–after (pre–post) studies with no control group, and case series studies [27].

Statistical analysis

Pooled proportions meta-analysis

The primary outcome was rate of relapse during pyrimethamine-based maintenance therapy. The secondary outcome was rate of relapse with continuous (daily) or intermittent (two to three times per week) pyrimethamine-based therapy. For each outcome, rates in each study were transformed using the Freeman-Tukey

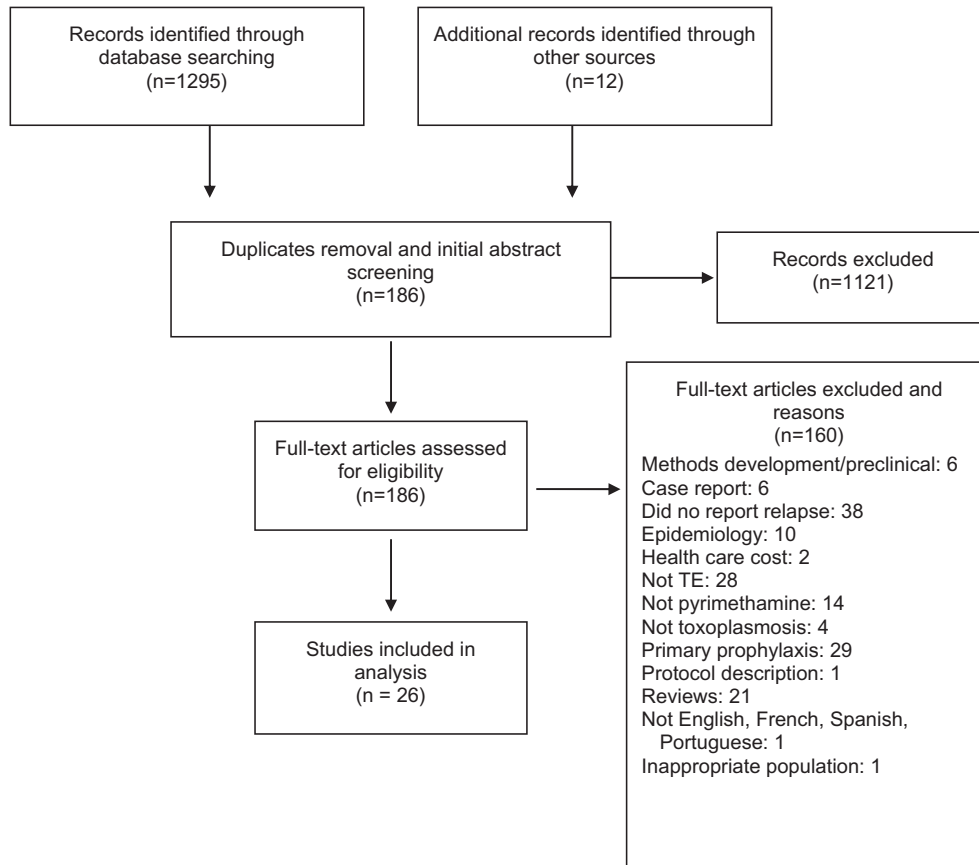


Figure 1. Search flow diagram.

double arcsine method. This transformation stabilizes the variance estimates, which is important for proportions close to 0 or 1 [28]. Rates were then pooled using both fixed-effect and random-effects meta-analysis models. The fixed-effect model was performed using the inverse variance method and the random-effects model using the DerSimonian and Laird method, with the estimate of heterogeneity taken from the inverse variance model [29]. Heterogeneity across studies was assessed by quantifying I^2 inconsistency scores [30]. I^2 describes the percentage variation across studies attributed to heterogeneity rather than chance. Low I^2 values ($\leq 50\%$) suggest limited heterogeneity between studies, and I^2 values > 50 to 75% indicate moderate heterogeneity. Values $> 75\%$ suggest high heterogeneity and the need to consider estimates from random-effects models [30]. The analysis was performed using StatsDirect (Altrincham, UK) statistical program version 2.8.0 (27 October 2013). For ease of interpreting, the reported proportions were converted to percentages. Because the studies are non-comparative, we assessed active treatment arms independently; therefore, no comparative statistics were performed and only the combined relapse rate is reported.

Bias of the results was evaluated by the funnel plot and the Egger's test [31,32]. In the absence of bias, the graph is a symmetrical inverted funnel, and if there is bias, the funnel plot will appear asymmetrical [31,32]. Reporting bias was assessed for the pre-HAART analysis,

but not the post-HAART analysis as the minimum studies for this analysis should be > 10 ; below this value, the test power is usually too low to distinguish chance from real asymmetry [33].

Results

Search results

Of 1,307 studies initially identified, 1,121 were excluded following the 'removal of duplicates and an initial abstract screen' (Figure 1). After full-text review, an additional 160 were removed for only describing methodology or a study protocol, being a pre-clinical study, being a case report, not reporting relapse rates, being an epidemiology or health care cost study, not evaluating TE, not administering pyrimethamine-based regimens, not investigating toxoplasmosis, describing finding for primary prophylaxis of TE, being a review article, not being published in English, French, Spanish, or Portuguese, or evaluating an inappropriate patient population.

Study characteristics

Twenty-six studies were included in the analysis with 1,596 patients (Tables 1 and 2). Twenty reported findings from studies that were performed up to 1996 ($n = 1,228$) (Table 1) [34–51], and six described results of studies performed after 1996 ($n = 368$) (Table 2) [52–57].

Table 1. Summary of study characteristics performed until 1996.

Author	Study Design	Demographics	Pyrimethamine-based therapy	Acute	Maintenance	No. patients evaluated for relapse, <i>n</i>	Relapse, <i>n</i> (%)	Follow-up
<i>Randomized studies</i>								
Katlama (1996)	Randomized prospective open-label	<i>N</i> = 299 <i>PC</i> (<i>n</i> = 152) Male: 133 (88%) Mean age: 34 yrs <i>PS</i> (<i>n</i> = 147) Male: 129 (88%) Mean age: 33 yrs	<i>PS</i> PYR (50 mg daily) plus SDZ (4 g daily in four divided doses) plus folinic acid (min 50 mg/wk)	<i>PS</i> PYR (50 mg daily) plus SDZ (4 g daily in four divided doses) plus folinic acid (min 50 mg/wk) Acute therapy was given for six weeks.	<i>PS</i> PYR (25 mg/day) plus SDZ (500 mg four times/day) plus folinic acid 50 mg weekly <i>PC</i> PYR (25 mg/day) plus CLI (300 mg four times/day) plus folinic acid 50 mg weekly Maintenance therapy was life-long.	<i>PS</i> : 83 <i>PC</i> : 92	<i>PS</i> : 17 (20.5%) <i>PC</i> : 35 (38.0%)	89 to >130 weeks
Podzamczar (1995)	Randomized open-label	<i>N</i> = 105 <i>Daily</i> : (<i>n</i> = 60) Male: 40 (66.7%) Age: 34 yrs <i>Twice weekly</i> : (<i>n</i> = 45) Male: 34 (75.6%) Age: 32 yrs	<i>PS</i> PYR (50 mg daily) plus clindamycin (2.4 g daily in four divided doses) plus folinic acid (min 50 mg/wk) Acute therapy was given for six weeks. <i>n.a.</i>	<i>Daily PS</i> PYR (25 mg/day) plus SDZ (500 mg four times per day) and folinic acid (15 mg/day). <i>Intermittent PS</i> PYR (25 mg/day) twice weekly plus SDZ (500 mg four times per day) and folinic acid (15 mg/day).	105	Total: 11 (10.4%) <i>Intermittent</i> : 19.5 per 100 patient-yrs <i>Daily</i> : 4.4 per 100 patient-yrs <i>PS</i> : 0 TMP-SMX: 1 (2.7%)	Total: 11 (10.4%) <i>Intermittent</i> : 19.5 per 100 patient-yrs <i>Daily</i> : 4.4 per 100 patient-yrs <i>PS</i> : 0 TMP-SMX: 1 (2.7%)	median 11 months
Torre (1998)	Randomized, prospective, pilot	<i>N</i> = 77 <i>PS</i> (<i>n</i> = 37) Male: 29 (78.4%) Mean age: 32.4 yrs <i>TMP-SMX</i> (<i>n</i> = 40) Male: 28 (70%) Mean age: 34.0 yrs	<i>PS</i> PYR (50 mg/day) plus SDZ (60 mg/kg/day) plus folinic acid (10 mg/day) <i>TMP-SMX</i> TMP (10 mg/kg/day BID) plus SMX (50 mg/kg/d BID) TX was for 30 days	<i>PS</i> PYR (50 mg/day) plus SDZ (60 mg/kg/day) plus folinic acid (10 mg/day) <i>TMP-SMX</i> TMP (10 mg/kg/day BID) plus SMX (50 mg/kg/d BID) TX was for 30 days	Same regimens as above but at half of the original dosage given for three months	<i>PS</i> : <i>n</i> = 35 TMP-SMX: <i>n</i> = 37	<i>PS</i> : 0 TMP-SMX: 1 (2.7%)	3 months
<i>Prospective studies</i>								
Bouree (1997)	Prospective observational	<i>N</i> = 60 Male: 46 (76.7) Mean age: 40 yrs. <i>N</i> = 24 Male: 19 (79.2%) Age, range: 23–53 yrs	<i>PS</i> PYR (100 mg/day) plus SDZ (6 g/day) plus folinic acid (50 mg/day) <i>TMP-SMX</i> TMP-SMX (40 mg/kg/day) or TMP-SMX (120 mg/kg/day) Folinic acid (15 mg/day) was also given TX duration 25 day	<i>PS</i> PYR (100 mg/day) plus SDZ (6 g/day) plus folinic acid (50 mg/day) <i>TMP-SMX</i> TMP-SMX (40 mg/kg/day) or TMP-SMX (120 mg/kg/day) Folinic acid (15 mg/day) was also given TX duration 25 day	<i>PS</i> PYR (100 mg/day) plus SDZ (6 g/day) plus folinic acid (50 mg/day) <i>PYR±SMX</i> Sulfamethoxypyrazine (500 mg) plus PYR (25 mg) twice weekly Same as acute	60 24 12	11 (18.3%) 4 (17%) 0	7 years n.r. n.r.
Leport (1987)	Prospective observational	<i>N</i> = 12 Male: 11 (91.7%) Age, range: 20–47 yrs	<i>PS</i> PYR (average 50 mg [range 15–100 mg] plus SDZ (average 4 g [range 2–6 g], for 30 days. Folinic acid (range, 5 to 50 mg/day) was also administered. The 12th patient died during TX	<i>PS</i> PYR (average 50 mg [range 15–100 mg] plus SDZ (average 4 g [range 2–6 g], for 30 days. Folinic acid (range, 5 to 50 mg/day) was also administered. The 12th patient died during TX	Same as acute	12	0	n.r.

Table 1. (Continued).

Author	Study Design	Demographics	Pyrimethamine-based therapy	Acute	Maintenance	No. patients evaluated for relapse, <i>n</i>	Relapse, <i>n</i> (%)	Follow-up
Ferrer (1996)	Retrospective	<i>N</i> = 63 Male: 46 (73%) Mean age for both: 21–65 years <i>PS</i> (<i>n</i> = 49) <i>PC</i> (<i>n</i> = 14)		<p><i>PS</i> PYR (50–75 mg/day) plus SDZ (4 g/day) plus folinic acid (15 mg/day) QID PO for 4–6 weeks</p> <p><i>PC</i> PYR (50–75 mg/day) plus folinic acid (15 mg/day) QID PO for 4–6 weeks plus CLI (600 mg/q6 h) PO or IV Pts w/ intracranial hypertension and perilesional edema: Dexamethasone (12–16 mg/day)</p>	<p><i>PS</i> PYR (25 mg/day) plus SDZ (2 g/day)</p> <p><i>PC</i> PYR (25 mg/day) plus CLI (1200 mg/day)</p>	63	Total: 19 (30.2%) <i>PS</i> : 12 <i>PC</i> : 7	n.r.
Foppa (1991)	Retrospective	<i>N</i> = 14 Male: 13 (92.9%) Age, range: 29–39 yrs		<p><i>PC</i> PYR (100 mg loading dose, followed by 50 mg/d) plus CLI (600–900 mg orally TID) plus folinic acid (15 mg/day) for 6–8 weeks</p> <p>Duration of acute therapy was from 6 to 8 weeks</p>	<i>PC</i> PYR (25 mg/d) plus CLI (300 mg QID or 450 mg TID) plus folinic acid (15 mg/day)	14	0	Mean 7.7 months
Gonzalez-Clemente (1990)	Retrospective	<i>N</i> = 57		<p><i>PS</i> PYR 5–100 mg for one day followed by 25–50 mg/day, SDZ 75 mg/kg/day divided into four doses, plus folinic acid (10–20 mg/day)</p> <p><i>PC</i> If patient had sulfa allergy, clindamycin (600 mg/every 6 h) was substituted for SDZ. Acute treatment was for 3 to 6 weeks</p>	<p><i>PS</i> PYR 5–100 mg for one day followed by 25–50 mg/day, SDZ 75 mg/kg/day divided into four doses, plus folinic acid (10–20 mg/day) two days per week (Tuesday and Friday)</p> <p><i>PC</i> If patient had sulfa allergy, clindamycin (600 mg/every 6 h) was substituted for SDZ</p>	<p><i>PS</i>: <i>n</i> = 15 <i>PC</i>: <i>n</i> = 10</p>	<p><i>PS</i>: 0 <i>PC</i>: 4 (40%)</p>	<p><i>PS</i>: Median 12.3 months <i>PC</i>: Median 13.3 months</p>
Leport (1988)	Retrospective chart review	<i>N</i> = 35 Male: 33 (94.3%) Mean age: 37.0 yrs		<p><i>PS</i> PYR (loading doses 100–200 mg for first 1–2 days, followed by 50–100 mg/day) plus SDZ (2–6 g/day) plus folinic acid (5–50 mg/day)</p>	<i>PS</i> PYR (25–50 mg/day) plus SDZ (1–4 g/day) plus folinic acid (5–50 mg/day)	24	6 (25%)	Mean 6 months
Leport (1991)	Retrospective	<i>N</i> = 35		n.a.	<i>PS</i> (<i>n</i> = 13) ^a PYR (25–100 mg/day) plus SDZ (2–4 g/day) <i>PC</i> (<i>n</i> = 20) ^a PYR (25–100 mg/day) plus CLI (450–1200 mg/day) <i>PS</i> (<i>n</i> = 11) ^a PYR 50–100 mg/day <i>PS</i> Same as acute dosage but given only 2 days/wk (Tues and Fri)	35	Total: 6 (17%) <i>PC</i> : 4 <i>PYR</i> : 1 <i>PS</i> : 1	Mean 13 months
Pedrol (1990)	Retrospective	<i>N</i> = 43 Male: 35 (81.4%)		<p><i>PS</i> PYR 50–75 mg loading dose, followed by 25 mg/day plus SDZ 75 mg/day plus folinic acid (10–20 mg/day)</p> <p>Acute treatment was for 3 weeks</p>	<i>PS</i> PYR 50–100 mg/day	12	6 (50.0%)	10.3–13.7 months
Ragnaud (1993)	Retrospective	<i>N</i> = 73 Sex ratio: 2.8:1 Mean age: 36.2 yrs		<p><i>PS</i> PYR (100–200 mg/day for 1–3 days followed by 75 mg/day) plus SDZ (4–8 g/day) plus folinic acid (10–50 mg/day)</p>	<i>PS</i> PYR 50 mg / day plus SDZ 2 to 3 g / day <i>PC</i> PYR 50 mg/day plus CLI 1.2 g/day	60	12 (20%)	8 months

Renold (1992)	Retrospective chart review	N = 86 Male: 69 (80%) Mean age: 36.4 yrs	PS PC	PS PC	PS PC	82	16 (19.5%)	n.r.
Acute treatment was defined as antitoxoplasma drugs received within 42 days of diagnosis								
Maintenance therapy was calculated from the 42nd day following diagnosis to death, loss to follow-up, relapse, or December 31, 1990, or whichever came first.								

^an indicates number of courses. Forty-four treatment courses were evaluated in 35 patients.

BID: twice daily; CLI, clindamycin; IV, intravenous; LEU, leucovorin; n.a., not applicable; n.r., not reported; PC, pyrimethamine plus clindamycin; PK, pharmacokinetic; PS, pyrimethamine plus sulfadiazine; PSC, pyrimethamine plus sulfadiazine, plus clindamycin; PYR, pyrimethamine; QID, four times/day; SDX, sulfadoxine; SDZ, sulfadiazine; SMP, sulphamethoxypyrazine; SMX, sulfamethoxazole; SPY, spiramycin; TMP, trimethoprim; TMP-SMX, trimethoprim-sulfamethoxazole (co-trimoxazole); TX, treatment.

Study designs differed across both pre- and post-HAART studies. The pre-HAART studies included three randomized, six prospective, and 11 retrospective studies (Table 1), and the post-HAART studies included two randomized, two prospective, and two retrospective studies (Table 2). All studies included patients with AIDS or infected with HIV and who had received maintenance therapy for TE.

Among the studies, the pyrimethamine-based treatment regimens were heterogeneous, both for acute and maintenance therapy (Tables 1 and 2). In the pre- and post-HAART groups, the most common therapy both for acute and maintenance therapy was the combination of pyrimethamine and sulfadiazine. Other investigated pyrimethamine-based regimens included clindamycin, sulphamethopyrazine, sulfadoxine, clarithromycin, and atovaquone. Four studies in the pre-HAART group [41,42,45,47] and two in the post-HAART group [52,55] administered pyrimethamine alone for maintenance therapy. Most studies co-administered folinic acid (leucovorin) with pyrimethamine for acute therapy; only four did not report co-administering folinic acid with pyrimethamine – three in the pre-HAART group [35,58,59] and one in the post-HAART group [52]. Of the included studies, 11 did not report co-administering folinic acid with pyrimethamine during maintenance therapy [34,35,38,41,45,47,49,51,52,54,60].

The follow-up for pre-HAART studies ranged from 3 months to 7 years and for the post-HAART studies from 4 to 22 months. For the pre-HAART studies, the rate of relapse ranged from 0 to 67% and for the post-HAART studies ranged from 0 to 12.9%.

Quality assessment

The majority of included studies obtained scores of medium-to-low quality due the varied trial designs. Despite the overall quality assessment, the relapse data reported were of sufficient quality for inclusion in the proportions analysis.

Meta-analysis

The pooled proportions test for pyrimethamine-based therapy in pre-HAART studies indicated a relapse rate of 19.2% and 18.9% from the fixed-effects and random-effects models, respectively (Table 3 and Figure 2 and Figure 3). In the post-HAART period, the pooled proportions relapse rate was 11.1%, and was the same from both the fixed- and random-effect models due to limited heterogeneity in the data ($I^2 = 0\%$) (Table 3 and Figure 4).

Six studies reported relapse rates of patients who received intermittent pyrimethamine-based therapy (two to three times per week); five pre-HAART [40,43,45,49,61] and one post-HAART [56]. We evaluated the rate of relapse in these studies compared with studies with continuous (daily) dosing. The relapse

Table 2. Summary of study characteristics after 1996.

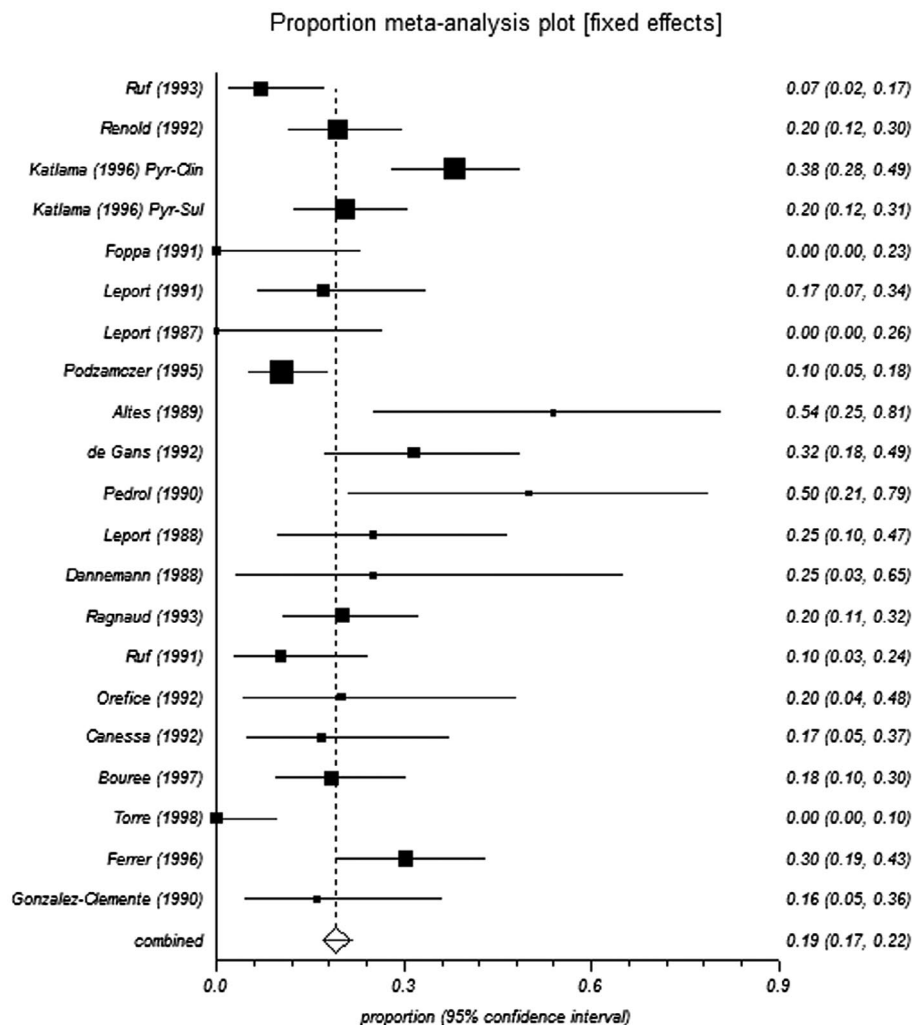
Author	Study design	Demographics	Pyrimethamine-based therapy		No. patients evaluated for relapse, <i>n</i>	Relapse, <i>n</i> (%)	Follow-up	
			Acute	Maintenance				
Randomized studies Chirgwin (2002)	Randomized, open-label, phase 2	<i>N</i> = 39 <i>ATO</i> ± <i>PYR</i> (<i>n</i> = 28) Male: 22 (79%) Median age: 35 yrs <i>ATO</i> ± <i>SDZ</i> (<i>n</i> = 12) Male: 10 (83%) Median age: 37 yrs	<i>ATO</i> ± <i>PYR</i> <i>ATO</i> (1500 mg BID) plus <i>PYR</i> (200 mg loading dose, followed by 75 mg/day) plus <i>LEU</i> 10 mg daily	42 weeks after acute treatment with same treatment regimen as acute therapy	15	1 (6.7%)	48 weeks	
			<i>ATO</i> ± <i>SDZ</i> <i>ATO</i> (1500 mg BID) plus <i>SDZ</i> (1500 mg QID) plus <i>LEU</i> 10 mg/day.					
			Acute treatment was administered for six weeks n.a.					
Podzamczar (2000)	Randomized open-label	<i>N</i> = 124 <i>Daily</i> (<i>n</i> = 58) Male/female ratio: 3.5 Age: 35.7 yrs <i>Thrice weekly</i> (<i>n</i> = 66) Male/female ratio: 5.0 Age: 34.9 yrs.		<i>PS</i> <i>Daily Regimen</i> : <i>PYR</i> (25 mg/day) plus <i>SDZ</i> (1 g BID) plus folinic acid (15 mg/day) <i>PS</i> <i>Three times/wk regimen</i> : <i>PYR</i> (50 mg/day) plus (<i>SDZ</i> : 1 g BID) plus folinic acid (15 mg)/day <i>PYR</i> : 50 mg/day or 37.5 mg/day depending on body weight plus folinic acid (7.5 mg/day) <i>PS</i> <i>PYR</i> + <i>SDZ</i> + folinic acid	124	Total: 16 (12.9%) <i>Daily</i> : 14.9 episodes per 100 patient yrs <i>Intermittent</i> : 14.1 episodes per 100 patient yrs	Median 11 months	
Prospective studies Langmann (2004)	Prospective	<i>N</i> = 6 (3 received secondary prophylaxis) Age, range: 35–59 yrs <i>N</i> = 55 Male: 33 (60%) Mean age: 36 yrs	n.a.		3	0	Mean 22 ± 13 months	
Vidal (2005)	Prospective longitudinal		<i>PS</i> <i>PYR</i> + <i>SDZ</i> + folinic acid		46	2 (4%)	One year from diagnosis	
Retrospective study Arendt (1999)	Retrospective	<i>N</i> = 106 Male: 101 (95.3%) Mean age: 40.4 yrs <i>P</i> ± <i>SND</i> (<i>n</i> = 98) <i>PC</i> (<i>n</i> = 6)	<i>P</i> ± <i>SND</i> <i>PYR</i> (75 mg/day) plus sulfonamides (2 g/day) or clindamycin (2400 mg/day). <i>PC</i> <i>PYR</i> (75 mg/day) plus clindamycin (2400 mg/day). <i>PS</i> <i>PYR</i> + <i>SDZ</i> + leucovorin	<i>PYR</i> : 75 mg/day	106	13 (12%)	n.r.	
Davarpanah (2007)	Retrospective	<i>N</i> = 38 Male: 30 (78.9%) Age: 38 yrs		<i>PS</i> <i>PYR</i> + <i>SDZ</i>	4	0	n.r.	

ATO, atovaquone; LEU, leucovorin; n.a., not applicable; n.r., not reported; PC, pyrimethamine plus clindamycin; PS, pyrimethamine plus sulfadiazine; P + SND, pyrimethamine plus a sulfonamide; PYR, pyrimethamine; SDZ, sulfadiazine.

Table 3. Pyrimethamine-based maintenance therapy: relapse rates in pre-HAART and post-HAART period.

	Proportions Fixed-effect	Proportions Random-effect	I^2
Pre-HAART	0.192 (95% CI = 0.167 to 0.218)	0.189 (95% CI = 0.137 to 0.247)	76.4% (95% CI = 62.8 to 83.5%)
Post-HAART	0.111 (95% CI = 0.078 to 0.149)	0.111 (95% CI = 0.078 to 0.149)	0% (95% CI = 0 to 61%)
Intermittent Pre-HAART	0.209 (95% CI = 0.139 to 0.287)	0.256 (95% CI = 0.104 to 0.445)	72.9% (95% CI = 17.9 to 86.7%)
Intermittent Post-HAART ^a	–	–	–
Continuous Pre-HAART	0.187 (95% CI = 0.161 to 0.214)	0.173 (95% CI = 0.119 to 0.234)	77.5% (95% CI = 64.4 to 84.3%)
Continuous Post-HAART	0.105 (95% CI = 0.069 to 0.146)	0.105 (95% CI = 0.069 to 0.146)	0% (95% CI = 0 to 61%)

^aAnalysis was not performed as only one study in the post-HAART timeframe evaluated intermittent pyrimethamine-based therapy.
CI, confidence interval; I^2 , inconsistency.

**Figure 2.** Forest plot of fixed for analysis of pre-HAART studies.

rate with intermittent pyrimethamine-based therapies in pre-HAART studies was 20.9% from the fixed-effect and 25.6% from the random-effect models, and for continuous therapy was 18.7–17.3%, respectively (Table 3). The rates for continuous therapy for post-HAART were 10.5%. Intermittent therapy was not analyzed in the post-HAART studies as only one study [56] evaluated this type of treatment regimen.

Analysis of reporting bias

Funnel plot analysis was performed for the pre-HAART studies (Figure 5). The analysis indicated the presence of reporting bias as indicated by significant funnel plot

asymmetry (Egger bias: 3.12386 (95% CI = 1.196098 to 5.051621); $P = 0.0031$).

Discussion

This systematic review and meta-analysis assessed the incidence of TE relapse following pyrimethamine-based therapy in patients with HIV or AIDS. The analysis found that the incidence of relapse was higher in pre-HAART (19.2% for fixed effect model and 18.9% for random effect model) compared with post-HAART (11.1% for both fixed- and random-effect models). The drop in the incidence of relapse with the use of HAART therapy is consistent with the impact of HAART on the immune

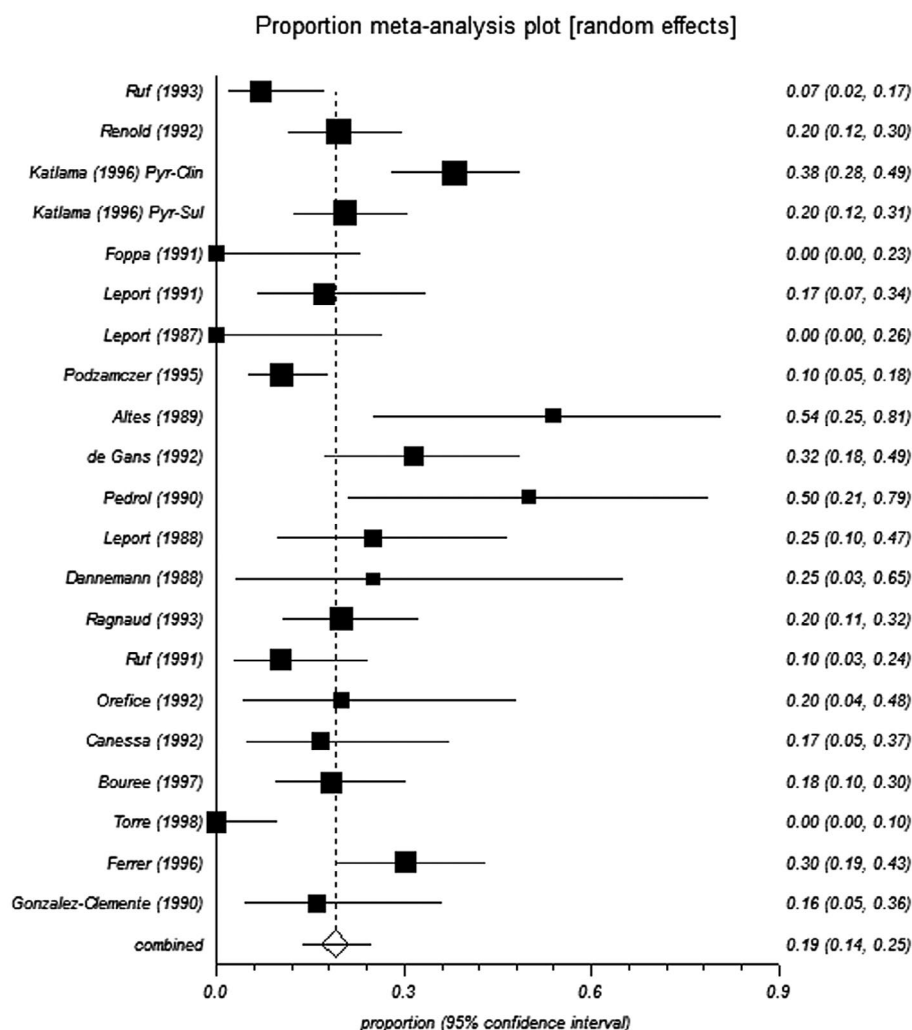


Figure 3. Forest plot of random effect model for analysis of pre-HAART studies.

status of patients with HIV or AIDs. To our knowledge, this is the first meta-analysis to evaluate the rate of relapse of TE in HIV-infected or AIDS patients, and specifically illustrates the benefits of HAART therapy for preventing relapse.

Relapse during maintenance therapy may result from a number of causes including treatment efficacy, dosing regimen, patient characteristics, and treatment adherence. The importance of the dosing regimen is indicated by our results that suggest intermittent therapy administered two to three times per week was associated with a higher relapse rate compared with continuous therapy, and supports the NIH Guidelines of daily dosing of pyrimethamine plus sulfadiazine for maintenance treatment [17].

The analysis described here focused on pyrimethamine-based therapy for the prevention of relapse due to the extensive history of the use of pyrimethamine in the clinic and the significant number of observational and retrospective clinical studies that have evaluated the effect of pyrimethamine-based therapy in treating TE. Much less-published information is available regarding the use of other off-label treatments for TE, such as the trimethoprim-sulfamethoxazole (TMP-SMX)

combination. A similar search identified only five studies that evaluated TMP-SMX for maintenance therapy and reported relapse of HIV-infected patients with TE [48,62–65], four of which were performed post-1996 [62–65]. Across the five studies, the rate of TE relapse ranged from 3 to 39%.

Evidence from randomized controlled trials serve as the gold-standard for studies to be included in meta-analyses to reduce bias in the results. However, challenges exist in applying randomized control trial results to the real-world clinical setting due to differences between subjects recruited and the types of patients encountered in clinical practice [66]. We included both prospective observational and retrospective studies due to the shortage of available randomized studies with pyrimethamine relative to the number of other study designs in TE. The inclusion of these studies in our analysis broadens the range of evidence and may improve the generalizability of the findings. This approach is consistent with other health service researchers who have included observational study designs in meta-analyses, and has been applied in other disease areas [67,68].

The analysis described here has several limitations that are worth considering. Significant heterogeneity

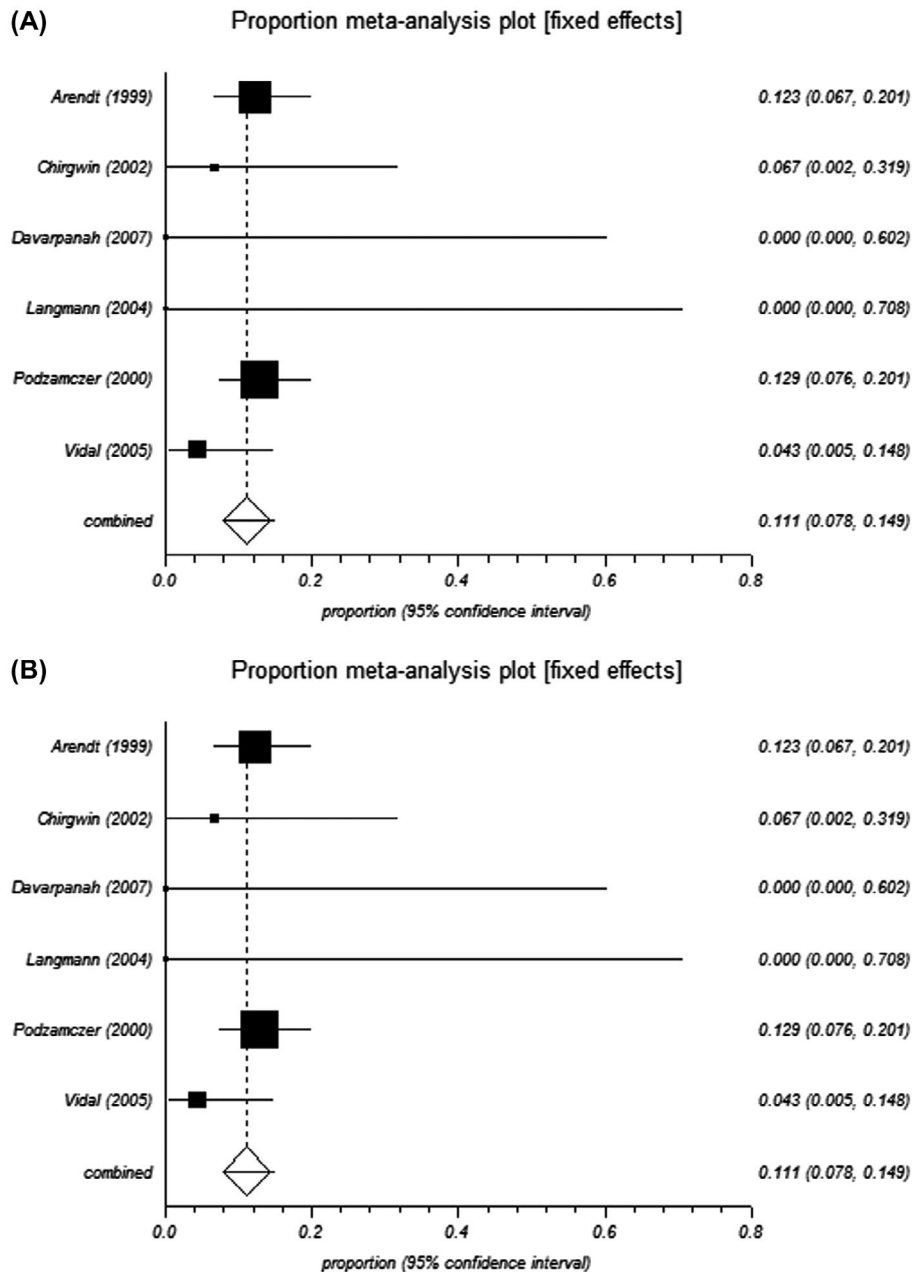


Figure 4. Forest plot of (A) fixed and (B) random effect model for analysis of post-HAART studies.

across studies was present with respect to study design, dosing regimen, and definition of relapse, and the majority of studies were of medium-to-low quality. Also, the size of the patient population for the post-HAART analysis was small. Furthermore, not all patients were receiving HAART therapy for various reasons, and the results were not presented by HAART precluding an assessment of how this would influence results. Although, based on the analysis described here it is likely to have diminished effectiveness and increased likelihood of relapse. Reporting bias was also present which may have confounded the results. In addition, duration of follow-up varied between studies which could influence the rate of relapse. It is also important to emphasize that the proportions reported here are independently evaluated;

therefore, it was not feasible to infer whether differences in relapse rates reported here are significantly different.

This study comprehensively evaluated the relapse rate of TE in patients with HIV or AIDS who received pyrimethamine-based maintenance therapy. The findings indicate that the incidence of relapse decreased after the introduction of HAART. The reduction in relapse following the introduction of HAART is consistent with the ability of HAART to control HIV infection. In addition, this study suggests that daily pyrimethamine-based maintenance therapy is likely more efficacious in preventing TE relapse compared with intermittent therapy. These findings have important medical and cost implications as relapse may affect a patient's prognosis and healthcare resource utilization, and result in additional healthcare expenditure.

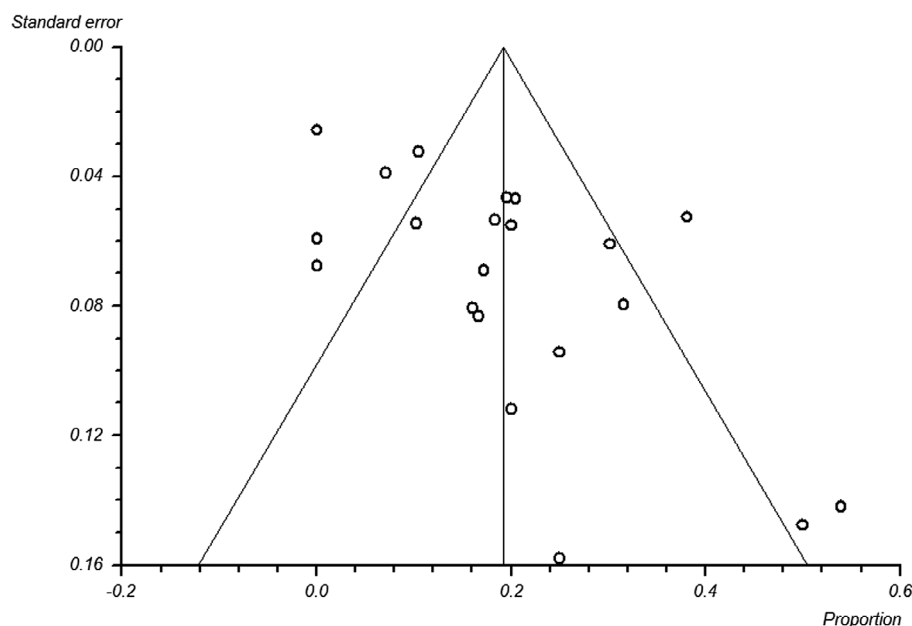


Figure 5. Funnel plot analysis of reporting bias.

Acknowledgment

The authors would like to thank Melissa Giraldo for assisting with the literature search, and Julio Casoy, MD and Rubben Ben-Harari, PhD for critically reading and reviewing the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Turing Pharmaceuticals.

References

- [1] Holland GN. Ocular toxoplasmosis: a global reassessment. Part I: epidemiology and course of disease. *Am J Ophthalmol*. 2003;136(6):973–88.
- [2] Dalimi A, Abdoli A. Latent toxoplasmosis and human. *Iran J Parasitol*. 2012;7(1):1–17.
- [3] Parasites – Toxoplasmosis (Toxoplasma infection): Resources for Health Professionals: Centers for Disease Control and Prevention; [Page last updated 2015 December 14].
- [4] Shin DW, Cha DY, Hua QJ, et al. Seroprevalence of *Toxoplasma gondii* Infection and characteristics of seropositive patients in general hospitals in Daejeon, Korea. *Korean J Parasitol*. 2009;47(2):125–30.
- [5] Xiao Y, Yin J, Jiang N, et al. Seroepidemiology of human *Toxoplasma gondii* infection in China. *BMC Infect Dis*. 2010;10:4.
- [6] Kamani J, Mani AU, Egbu GO, et al. Seroprevalence of human infection with *Toxoplasma gondii* and the associated risk factors, in Maiduguri, Borno state, Nigeria. *Ann Trop Med Parasitol*. 2009;103(4):317–21.
- [7] Swai ES, Schoonman L. Seroprevalence of *Toxoplasma gondii* infection amongst residents of Tanga district in North-East Tanzania. *Tanzan J Health Res*. 2009;11(4):205–9.
- [8] Fromont EG, Riche B, Rabilloud M. Toxoplasma seroprevalence in a rural population in France: detection of a household effect. *BMC Infect Dis*. 2009;9:1217.
- [9] Dubey JP, Lago EG, Gennari SM, et al. Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease, and epidemiology. *Parasitology*. 2012;139(11):1375–424.
- [10] Derouin F, Pelloux H. Parasitology ESGoC. Prevention of toxoplasmosis in transplant patients. *Clin Microbiol Infect*. 2008;14(12):1089–101.
- [11] Montoya JG, Liesenfeld O. Toxoplasmosis. *Lancet*. 2004;363(9425):1965–76.
- [12] Pereira-Chioccia VL, Vidal JE, Su C. *Toxoplasma gondii* infection and cerebral toxoplasmosis in HIV-infected patients. *Future Microbiol*. 2009;4(10):1363–79.
- [13] Luft BJ, Remington JS. Toxoplasmic encephalitis in AIDS. *Clin Infect Dis*. 1992;15(2):211–22.
- [14] Grant IH, Gold JW, Rosenblum M, et al. *Toxoplasma gondii* serology in HIV-infected patients: the development of central nervous system toxoplasmosis in AIDS. *AIDS*. 1990;4(6):519–22.
- [15] Ruskin J, Remington JS. Toxoplasmosis in the compromised host. *Ann Intern Med*. 1976;84(2):193–9.
- [16] Vora NM, Holman RC, Mehal JM, et al. Burden of encephalitis-associated hospitalizations in the United States, 1998–2010. *Neurology*. 2014;82(5):443–51.
- [17] Panel on antiretroviral guidelines for adults and adolescents; guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents. Washington, DC: Department of Health and Human Services; [updated 2015 October 19]. Available from: <https://aidsinfo.nih.gov/guidelines/html/4/adult-and-adolescent-oi-prevention-and-treatment-guidelines/322/toxo>.
- [18] Brackett CC, Singh H, Block JH. Likelihood and mechanisms of cross-allergenicity between sulfonamide antibiotics and other drugs containing a sulfonamide functional group. *Pharmacotherapy*. 2004;24(7):856–70.
- [19] Waxman S, Metz J, Herbert V. Defective DNA synthesis in human megaloblastic bone marrow: effects of homocysteine and methionine. *J Clin Invest*. 1969;48(2):284–9.

- [20] Martin-Blondel G, Alvarez M, Delobel P, et al. Toxoplasmic encephalitis IRIS in HIV-infected patients: a case series and review of the literature. *J Neurol Neurosurg Psychiatry*. 2011;82(6):691–3.
- [21] Wei HX, Wei SS, Lindsay DS, et al. A systematic review and meta-analysis of the efficacy of anti-*Toxoplasma gondii* medicines in humans. *PLoS One*. 2015;10(9):e0138204.
- [22] Yan J, Huang B, Liu G, et al. Meta-analysis of prevention and treatment of toxoplasmic encephalitis in HIV-infected patients. *Acta Trop*. 2013;127(3):236–44.
- [23] Hernandez AV, Thota P, Pellegrino D, et al. A systematic review and meta-analysis of the relative efficacy and safety of treatment regimens for HIV-associated cerebral toxoplasmosis: is trimethoprim-sulfamethoxazole a real option? *HIV Med*. doi: 10.1111/hiv.12402.
- [24] Rajapakse S, Chrisan Shivanthan M, Samaranayake N, et al. Antibiotics for human toxoplasmosis: a systematic review of randomized trials. *Pathog Glob Health*. 2013;107(4):162–9.
- [25] Palella FJ Jr, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV outpatient study investigators. *N Engl J Med*. 1998;338(13):853–60.
- [26] Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *J Clin Epidemiol*. 2009;62(10):e1–34.
- [27] National heart lung and blood institute study quality assessment tools. Available from: <https://www.nhlbi.nih.gov/health-pro/guidelines/in-develop/cardiovascular-risk-reduction/tools>
- [28] Freeman FM, Tukey JW. Transformations related to the angular and the square root. *Ann Math Stat*. 1950;21(4):607–11.
- [29] DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7(3):177–88.
- [30] Cochrane handbook for systematic reviews of interventions Available from: <http://training.cochrane.org/handbook>.
- [31] Sterne JA, Egger M. Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *J Clin Epidemiol*. 2001;54(10):1046–55.
- [32] Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997;315(7109):629–34.
- [33] Sterne JA, Sutton AJ, Ioannidis JP, Terrin N, Jones DR, Lau J, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ*. 2011;343:d4002.
- [34] Ruf B, Schürmann D, Bergmann F, et al. Efficacy of pyrimethamine/sulfadoxine in the prevention of toxoplasmic encephalitis relapses and pneumocystis carinii pneumonia in HIV-infected patients. *Eur J Clin Microbiol Infect Dis*. 1993;12(5):325–9.
- [35] Renold C, Sugar A, Chave JP, et al. Toxoplasma encephalitis in patients with the acquired immunodeficiency syndrome. *Medicine (Baltimore)*. 1992;71(4):224–39.
- [36] Katlama C, Wit S, O'Doherty E, et al. Pyrimethamine-clindamycin vs. pyrimethamine-sulfadiazine as acute and long-term therapy for toxoplasmic encephalitis in patients with AIDS. *Clinical Infect Dis*. [Internet]. 1996; 22(2):268–75. Available from: <http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/960/CN-00130960/frame.html>.
- [37] Foppa CU, Bini T, Gregis G, et al. A retrospective study of primary and maintenance therapy of toxoplasmic encephalitis with oral clindamycin and pyrimethamine. *Eur J Clin Microbiol Infect Dis*. 1991;10(3):187–9.
- [38] Leport C, Tournier C, Raguin G, et al. Long-term follow-up of patients with AIDS on maintenance therapy for toxoplasmosis. *Eur J Clin Microbiol Infect Dis*. 1991;10(3):191–3.
- [39] Leport C, Vilde JL, Katlama C, et al. Cerebral toxoplasmosis in immunosuppressed patients: diagnosis and treatment. *Ann Med Interne (Paris)*. 1987;138(1):30–3.
- [40] Podzamczar D, Miro JM, Bolao F, et al. Twice-weekly maintenance therapy with sulfadiazine-pyrimethamine to prevent recurrent toxoplasmic encephalitis in patients with AIDS. Spanish toxoplasmosis study group. *Ann Intern Med*. 1995;123(3):175–80.
- [41] Altes J, Salas A, Ricart C, et al. Cerebral toxoplasmosis in patients with AIDS. *Arch Neurobiol (Madr)*. 1989;52(Suppl 1):121–6.
- [42] de Gans J, Portegies P, Reiss P, et al. Pyrimethamine alone as maintenance therapy for central nervous system toxoplasmosis in 38 patients with AIDS. *J Acquir Immune Defic Syndr*. 1992;5(2):137–42.
- [43] Pedrol E, Gonzalez-Clemente JM, Gatell JM, et al. Central nervous system toxoplasmosis in AIDS patients: efficacy of an intermittent maintenance therapy. *AIDS*. 1990;4(6):511–8.
- [44] Leport C, Raffi F, Matheron S, et al. Treatment of central nervous system toxoplasmosis with pyrimethamine/sulfadiazine combination in 35 patients with the acquired immunodeficiency syndrome. Efficacy of long-term continuous therapy. *Am J Med*. 1988;84(1):94–100.
- [45] Dannemann BR, Israelski DM, Remington JS. Treatment of toxoplasmic encephalitis with intravenous clindamycin. *Arch Intern Med*. 1988;148(11):2477–82.
- [46] Ruf B, Pohle H. Role of clindamycin in the treatment of acute toxoplasmosis of the central nervous system. *Eur J Clin Microbiol Infect Dis*. 1991;10(3):183–6.
- [47] Orefice G, Carrieri PB, Chirianni A, et al. Cerebral toxoplasmosis and AIDS. Clinical, neuroradiological and immunological findings in 15 patients. *Acta Neurol*. 1992;14(4–6):493–502.
- [48] Torre D, Casari S, Speranza F, et al. Randomized trial of trimethoprim-sulfamethoxazole versus pyrimethamine-sulfadiazine for therapy of toxoplasmic encephalitis in patients with AIDS. *Antimicrob Agents Chemother*. 1998;42(6):1346–9.
- [49] Canessa A, Del Bono V, De Leo P, et al. Cotrimoxazole therapy of *Toxoplasma gondii* encephalitis in AIDS patients. *Eur J Clin Microbiol Infect Dis*. 1992;11(2):125–30.
- [50] Bouree P, Dumazedier D, Magdeleine C, et al. Cerebral toxoplasmosis and AIDS in martinique. *Med Trop (Mars)*. 1997;57(3):259–61.
- [51] Ragnaud JM, Morlat P, Dupon M, et al. Cerebral toxoplasmosis in AIDS. 73 cases. Clinical epidemiology group on AIDS in aquitania. *Presse Med*. 1993;22(19):903–8.
- [52] Arendt G, von Giesen HJ, Hefter H, et al. Long-term course and outcome in AIDS patients with cerebral toxoplasmosis. *Acta Neurol Scand*. 1999;100(3):178–84.
- [53] Chirgwin K, Hafner R, Leport C, et al. Randomized phase II trial of atovaquone with pyrimethamine or sulfadiazine for treatment of toxoplasmic encephalitis in patients with acquired immunodeficiency syndrome: ACTG 237/ANRS 039 Study. AIDS Clinical Trials Group 237/Agence Nationale de Recherche sur le SIDA, Essai 039. *Clinical Infect Dis*. [Internet]. 2002; 34(9):1243–50. Available from: <http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/407/CN-00379407/frame.html>.

- [54] Davarpanah M, Mehrabani D, Neirami R, et al. Toxoplasmosis in HIV/AIDS patients in Shiraz, southern Iran. *Iran Red Crescent Med J.* 2007;2007(1):22–7.
- [55] Langmann P, Schirmer D, Zilly M, et al. Drug monitoring of pyrimethamine during maintenance therapy of toxoplasmic encephalitis in patients with advanced HIV infection during HAART. *Med Sci Monit.* 2004;10(5): PI65–9.
- [56] Podzamczar D, Miro JM, Ferrer E, et al. Thrice-weekly sulfadiazine-pyrimethamine for maintenance therapy of toxoplasmic encephalitis in HIV-infected patients. Spanish toxoplasmosis study group. *Eur J Clin Microbiol Infect Dis.* 2000;19(2):89–95.
- [57] Vidal JE, Hernandez AV, De Oliveira ACP, et al. Cerebral toxoplasmosis in HIV-positive patients in Brazil: clinical features and predictors of treatment response in the HAART era. *AIDS Patient Care St.* 2005;19(10): 626–34.
- [58] Ragnaud J, Morlat P, Dupon M, et al. Relapse of brain toxoplasmosis in 25 AIDS patients. *Med Mal Infect.* 1993;23(11):791–5.
- [59] Walckenaer G, Leport C, Longuet P, et al. Recurrence of cerebral toxoplasmosis in 15 AIDS patients. *Ann Med Interne (Paris).* 1994;145(3):181–4.
- [60] Ferrer S, Fuentes I, Domingo P, et al. Cerebral toxoplasmosis in patients with human immunodeficiency virus (HIV) infection. Clinico-radiological and therapeutic aspects in 63 patients. *Anales de medicina interna (Madrid, Spain: 1984).* 1996;13(1):4–8.
- [61] Gonzalez-Clemente JM, Miro JM, Pedrol E, et al. Encephalic toxoplasmosis in patients with the acquired immunodeficiency syndrome. A clinico-radiological study and the therapeutic results in 78 cases. *Med Clin (Barc).* 1990;95(12):441–6.
- [62] Beraud G, Pierre-Francois S, Foltzer A, et al. Cotrimoxazole for treatment of cerebral toxoplasmosis: an observational cohort study during 1994–2006. *Am J Trop Med Hyg.* 2009;80(4):583–7.
- [63] Chaddha DS, Kalra SP, Singh AP, et al. Toxoplasmic encephalitis in acquired immunodeficiency syndrome. *J Assoc Physicians India.* 1999;47(7):680–4.
- [64] Duval X, Pajot O, Le Moing V, et al. Maintenance therapy with cotrimoxazole for toxoplasmic encephalitis in the era of highly active antiretroviral therapy. *Aids.* 2004;18(9):1342–4.
- [65] Smadja D, Fournier P, Cabre P, et al. Efficacy and good tolerance of cotrimoxazole as treatment of cerebral toxoplasmosis in AIDS. *Presse Med.* 1998;27(26):1315–20.
- [66] Rothwell PM. External validity of randomised controlled trials: “to whom do the results of this trial apply?”. *Lancet.* 2005;365(9453):82–93.
- [67] Shrier I, Boivin JF, Steele RJ, et al. Should meta-analyses of interventions include observational studies in addition to randomized controlled trials? a critical examination of underlying principles. *Am J Epidemiol.* 2007;166(10):1203–19.
- [68] Cox H, Ford N. Linezolid for the treatment of complicated drug-resistant tuberculosis: a systematic review and meta-analysis. *Int J Tuberc Lung Dis.* 2012;16(4):447–54.